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THE INFLUENCE OF POTASSIUM AND CHLORIDE IONS ON THE MEMBRANE POTENTIAL OF SINGLE MUSCLE FIBRES

BY A. L. HODGKIN AND P. HOROWICZ*

From the Physiological Laboratory, University of Cambridge

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The most widely accepted theory of the resting potential of muscle is that the electrical potential difference between the inside and outside of a muscle fibre arises from the concentration gradients of the potassium and chloride ions. If we follow Boyle & Conway (1941), the membrane is assumed to be permeable to K and Cl but to be impermeable or sparingly permeable to other ions. Since K is more concentrated inside and Cl is more concentrated outside, the interior of the fibre should be electrically negative to the external solution. If K and Cl are distributed passively, the concentration ratios and the membrane potential under equilibrium conditions ought to conform to the relation

$$\frac{[K]_o}{[K]_i} = \frac{[Cl]_i}{[Cl]_o} = \exp(VF/RT), \quad (1)$$

where $[]_o$ and $[]_i$ indicate concentrations outside and inside the fibre, V is the internal potential, F is Faraday's constant, R is the gas constant and T is the absolute temperature. When the external potassium concentration is less than 10 mM agreement with equation (1) is not perfect, but at higher concentrations the relation seems to hold with considerable accuracy (Adrian, 1956; Conway, 1957). However, it is only possible to calculate the membrane potential by equation (1) when the concentrations of K and Cl inside the fibre have come into equilibrium with those in the external solution. In order to deal with other situations one must know the relative conductances or permeabilities of the two ions. Information on this point can be obtained by measuring the effect on membrane potential of sudden changes in the concentrations of K or Cl. Such experiments are difficult to interpret unless the effect of changing the external solution can be determined in a time which is so short that there is no alteration in the internal concentration of Cl or K. The present observations were carried out with single muscle fibres, with an

* Present address; Department of Physiology, Washington University, St Louis, Missouri, U.S.A.

apparatus in which the external solution could be changed in a fraction of a second. The general conclusion is that the membrane potential is affected by both K and Cl. Under appropriate conditions, the membrane can be made to behave either as a potassium electrode or as a chloride electrode, but in most situations both ions contribute to the potential. When a fibre is equilibrated in Ringer's fluid the transport number of Cl is about 0.6, whereas that of K is about 0.3.

In addition to consolidating the conventional theory of the resting potential, the experiments with single fibres provided some new information which cannot easily be fitted into a simple physical picture. These observations, which relate to the rapid time course of the change in potential associated with a rise or fall in potassium concentration, will be deferred to a later paper. It is not thought that the questions raised by these results are likely to alter the main conclusions of the present article.

A preliminary account of some of the experiments described here was given at a meeting of the Physiological Society (Hodgkin & Horowicz, 1957).

METHODS

Single fibres from the semitendinosus muscles of English frogs (*Rana temporaria*) were used throughout the investigation. When the fibres were mounted in the apparatus they were stretched to $\frac{4}{3}$ of their slack length. The length in the apparatus was about 16 mm and the diameters of the fibres varied between 70 and 170 μ .

The main features of the recording cell are illustrated diagrammatically in Fig. 1. Single fibres were mounted in a channel in a Perspex cell, into which solutions of different composition could be introduced by a multiple tap. The tendon at one end of the fibre was gripped in a small Perspex clamp and the other tendon was connected to a transducer (RCA 5734). The fibre was suspended freely in the solution, except near the clamped end where it rested on a smooth glass pedestal which was thinly covered with petroleum jelly. Membrane potentials were recorded with an internal electrode of the Ling-Gerard type. The micro-electrode was left in position while the solution was changed, and alterations in membrane potential were recorded photographically on moving film. The micro-electrode was not left inside the fibre for more than about 2 min, and in measurements lasting for longer periods the potential was determined by successive impalements. This method required some practice and was only successful if the micro-electrode penetrated without appreciable dimpling. In the best experiments the fibre retained its excitability and remained free from opacities for many hours. However, as a rule it was not possible to make more than about ten successive impalements in any one fibre.

In the majority of experiments membrane potentials were recorded between an external micro-electrode which was always kept in the external solution and an internal micro-electrode which was periodically lowered into the fibre. Both electrodes were filled with 3M-KCl and had tip potentials of less than 5 mV (Adrian, 1956). The resistance of the internal electrode was 5–10 M Ω while that of the external electrode was 2–10 M Ω .

When potential differences were recorded between two micro-electrodes of low tip potential there was usually no need to correct for liquid junction potentials between the different test solutions. This was established by the following tests which were carried out in all experiments with solutions which give different junction potentials. (1) Both micro-electrodes were in the external solution and the potential difference between them was recorded photographically while the solutions used in the experiment were run through the cell. If both micro-electrodes were unaffected by junction potentials no change in potential occurred. There would also have been

no change if both electrodes had been affected by the junction potential to the same extent; this possibility was eliminated by the second and third tests. (2) The test was repeated with the potential difference recorded between the external micro-electrode and an agar-Ringer electrode. If the external micro-electrode was satisfactory, the standard junction potential was observed when the solution was changed: by standard junction potential is meant the junction potential expected if the micro-electrode were acting like a saturated KCl bridge. (3) This was a similar test to (2) but using the internal electrode and the agar-Ringer electrode; it did not give any

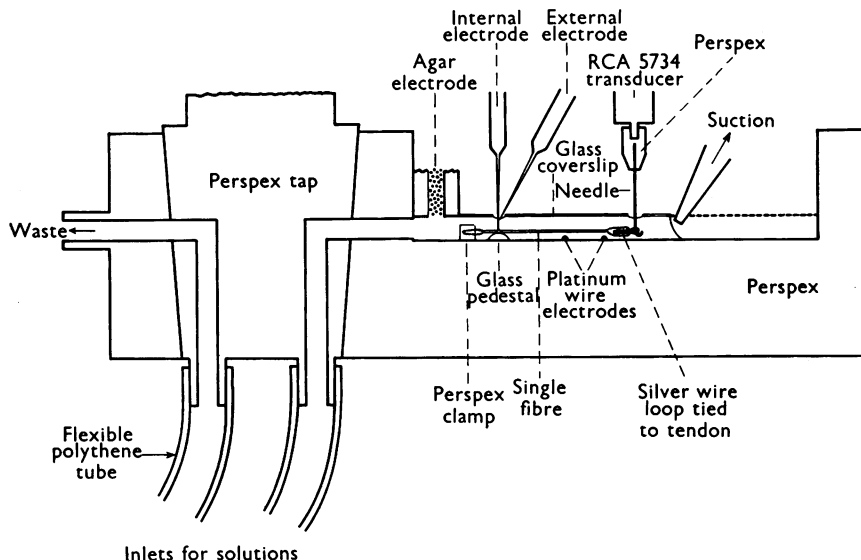


Fig. 1. Diagram of measuring cell. The multiple tap had six inlets, of which only two are shown. There were also six outlets, one to the cell and five to the 'waste'. The lines containing solutions were clamped when not in use but were always flushed into the 'waste' before being connected to the cell. Silver wires in the electrodes are not shown. The twisted end of the silver wire loop was bound to the tendon with a strand of silk thread which is not shown in the drawing. The drawing is approximately to scale, the length of the fibre being 1.6 cm.

new information but was a check of the first two tests. In the great majority of cases these tests showed that both electrodes were recording with negligible effects from junction potentials; corrections were made in a few cases in which the external electrode was found to be affected by the junction potential. When working with solutions which did not give appreciable junction potentials (e.g. solutions *A*, *B* and *C* in Table 1) it was sometimes convenient to use the agar-Ringer electrode instead of an external micro-electrode.

Solutions

Ringer's fluid. This was the same as that used by Adrian (1956); its composition is given opposite *A* in Table 1. The chloride concentration was 121.1 mM but in all subsequent tables a rounded figure of 120 mM has been employed.

Ringer's fluid with K replacing Na. An example is given opposite *B* in Table 1.

K-free Ringer's fluid; see solution *C*, Table 1.

Na-free solutions containing Cl. These are not listed in Table 1 but were similar to solutions *A*, *B* or *C* with choline replacing Na on a mole-for-mole basis.

Isotonic sulphate solutions with the same ionic strength as Ringer's fluid. Cl-free isotonic solutions, with K varying between 0 and 80 mM, were made by mixing solutions *D* and *E* in appropriate

proportions; since both these solutions were made up with concentrations calculated to give the same ionic strength as Ringer's fluid, there was no need to correct for changes in activity coefficient.

In order to make one litre of the solution *D* or *E*, 797 ml. of 10 mM- CaSO_4 was added to 203 ml. of the remaining ingredients, i.e. 80 ml. 0.5M- Na_2 or K_2SO_4 , 10 ml. 0.15M- Na phosphate buffer and 113 ml. 1M sucrose. The resulting concentration of 8 mM- Ca was chosen because it was the highest which could conveniently be got into solution. If the dissociation constant of CaSO_4 is taken as 5.3×10^{-3} mole/l. (Brink, 1954) the concentration of ionized calcium is found to be 1 mM instead of 1.8 mM in Ringer's fluid.

We are indebted to Professor A. V. Hill for suggesting that CaSO_4 should be added to sulphate solutions (see Hill & Howarth, 1957).

TABLE 1. Composition of solutions

Ref.	K^+	Cl^-	Na^+	Ca^{2+}	HPO_4^{2-}	H_2PO_4^-	SO_4^{2-}	Sucrose (m-mole/l. solution)	Relative tonicity	Relative ionic strength
	(mg ion/l. solution)									
<i>A</i>	2.5	121	120	1.8	2.15	0.85	—	—	1.0	1.0
<i>B</i>	10	121	112.5	1.8	2.15	0.85	—	—	1.0	1.0
<i>C</i>	0	121	122.5	1.8	2.15	0.85	—	—	1.0	1.0
<i>D</i>	0	0	83	8	1.08	0.43	48	113	1.0	1.0
<i>E</i>	80	0	3	8	1.08	0.43	48	113	1.0	1.0
<i>F</i>	10	30	83	6.5	1.35	0.54	36	85	1.0	1.0
<i>G</i>	2.5	30	90	6.5	1.35	0.54	36	85	1.0	1.0
<i>H</i>	190	0	3	9	1.08	0.43	104	—	1.0	2.3
<i>I</i>	2.5	214	212.5	1.8	2.15	0.85	—	—	1.75	1.7
<i>J</i>	95	214	120	1.8	2.15	0.85	—	—	1.75	1.7
<i>K</i>	95	0	98	6.3	1.08	0.43	101	168	1.75	2.3

A is Ringer's fluid. *B-G* are solutions of the same tonicity and ionic strength as *A* but with different [K] and [Cl]. *H* is an isotonic solution with high [K] and increased ionic strength. *I-K* are hypertonic solutions. In estimating tonicities and ionic strengths CaSO_4 was regarded as mainly un-ionized.

Isotonic potassium sulphate without sucrose. The composition of this solution is given against *H*. The potassium concentration was 190 mM, but since the ionic strength was greater than that in Ringer's fluid a correction must be made for the change in ionic strength. From the Debye-Hückel theory and tables of mean activity coefficients (Taylor, 1931), it is estimated that the activity coefficient of K in this solution was 0.87 times that in Ringer's fluid. A corrected concentration of $0.87 \times 190 = 165$ mM has been used in Table 2 and Fig. 4.

Solutions with constant [K] [Cl] product. Since Ringer's fluid contains 2.5 mM-K and 120 mM-Cl the [K] [Cl] product is 300 (mM)². In order to make up solutions with different K or Cl concentrations but with the same product, we mixed appropriate proportions of solutions *C*, *D* and *E* (Table 1). Thus to make solution *F* (10 mM-K 30 mM-Cl), one must take 25 parts *C*, 12.5 parts *E* and 62.5 parts *D*. Since each of the master solutions, *C*, *D* or *E* was isotonic with Ringer's fluid and had the same ionic strength as Ringer's fluid the resulting solution should be isotonic and should also have the same ionic strength as Ringer's fluid. There is therefore no need to correct for changes in activity coefficient. Solutions made up in this way had potassium concentrations of 5, 10, 30 or 75 mM and chloride concentrations of 60, 30, 10 or 4 mM. In order to extend the range, a solution containing 190 mM-K and 2.5 mM-Cl was made from solution *H*. After correcting for the change in activity coefficient the effective concentrations at the same ionic strength as Ringer's fluid were taken as 165 mM-K and 2.2 mM-Cl. The nominal [K] [Cl] product is 360 instead of 300 but the chloride concentration is so low that the precise value is unimportant.

Solutions of constant [K] [Cl] product without sulphate were made by replacing some of the NaCl in Ringer's fluid with an osmotically equivalent quantity of sucrose and adjusting the K and Cl concentrations to allow for change in ionic strength. The disadvantages of this method are that [K] cannot be made greater than [Cl] and that solutions of low ionic strength may have deleterious effects on muscle.

Solutions with normal $[K]_o$ and reduced $[Cl]_o$. These were made in the same way as the solutions with constant $[K] [Cl]$ product; an example of one of the solutions most often used is given opposite *G* in Table 1.

Hypertonic solutions. Solutions *I* and *J* in Table 1 were made by adding NaCl or KCl to Ringer's fluid; solution *K* is a chloride-free solution with the same osmotic pressure and potassium concentration as *J*.

RESULTS

Variations of $[K]_o$ and $[Cl]_o$ at constant product

According to the theory of Boyle & Conway (1941) there should be no movement of KCl across the fibre membrane if $[K]_o$ and $[Cl]_o$ are varied in a manner such that the product $[K]_o [Cl]_o$ is kept constant. Under these conditions the membrane potential should respond rapidly and reversibly to alterations in the K and Cl concentrations and its absolute value should be the same as the equilibrium potentials of the potassium and chloride ions, i.e.

$$V_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i}, \quad (2)$$

$$V_{Cl} = \frac{RT}{F} \ln \frac{[Cl]_i}{[Cl]_o}. \quad (3)$$

The record in Fig. 2 illustrates the effect of substituting a solution containing 10 mM-K 30 mM-Cl for Ringer's fluid (2.5 mM-K 120 mM-Cl). The potential takes up its new value in about 3 sec and the change in potential, which is reversible, is within 1 mV of that predicted by eqn. 1, 35 mV. There was no change in potential at b and d, when the cell was flushed with the solution already in the cell. This shows that the solution was adequately changed in one flush and that there was no flow artifact.

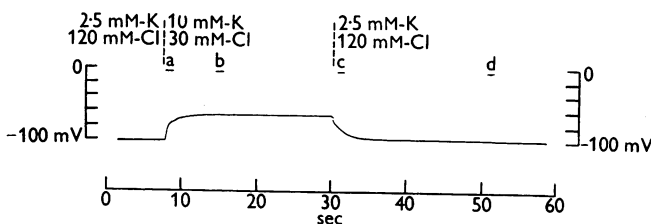


Fig. 2. Record illustrating the effect on membrane potential of a short application of 10 mM-K 30 mM-Cl to a fibre equilibrated in Ringer's fluid containing 2.5 mM-K 120 mM-Cl. The solutions, whose composition is given under *A* and *F*, Table 1, were isotonic and had the same ionic strength. At a Ringer's fluid was changed for the test solution, at b the test solution was flushed through a second time, at c Ringer's fluid was restored and at d it was flushed through a second time. In each case 2-3 ml. was flushed through the cell at about 2 ml./sec. The horizontal lines below the letters a-d mark the periods when solution was flowing through the cell. These lines were derived from the transducer output which was also recorded photographically but is not shown here. The scale gives the internal potential. There was no change in potential when both electrodes were outside the fibre. Temperature, 20° C; fibre *i*; diameter 145 μ .

Figure 3 gives the time course of the membrane potential during the experiment. Although there was some random variation between successive impalements, the membrane potential was established in a rapid and reversible manner and there was no drift or hysteresis of the type encountered when K was changed at constant $[Cl]_o$. The reversible nature of the changes is shown more clearly by Fig. 4 in which average potentials are plotted against $\log [K]_o$ or $-\log [Cl]_o$. The crosses give the potentials after equilibrating in each solution

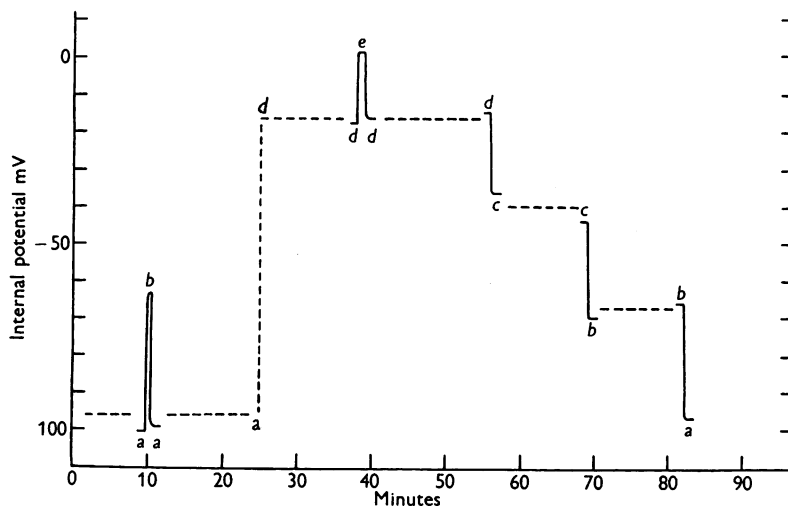


Fig. 3. Effect on membrane potential of solutions with a $[K][Cl]$ product of 300 (mM)^2 . The concentrations of K and Cl were:

	K (mM)	Cl (mM)
a	2.5	120
b	10	30
c	30	10
d	75	4
e	190	2.5

Solutions *a-d* had the same ionic strength as Ringer's fluid; the activity coefficient of K and Cl in solution *e* was estimated as 0.87 times that in Ringer's fluid. The continuous lines were drawn from photographic records with the micro-electrode in position. Broken lines were drawn at the average potential produced by each solution in this experiment. The fibre was impaled at different spots, roughly 50μ apart. A contracture lasting 10 sec occurred at 25 min when solution *d* was applied; the micro-electrode had not been inserted because a contracture was expected. Fibre *i*, as in Fig. 2.

for about 10 min; the other symbols give the potentials established 20–30 sec after a sudden increase or decrease in potassium concentration. The straight line represents the equation for a potassium or chloride electrode. The value of 140 mM for the internal potassium concentration agrees with the figure of 139 ± 2 m-mole/kg fibre water, given by Adrian (1956) for the potassium concentration in the sartorius muscle and with the value of 139 ± 7 m-mole/kg fibre water obtained on fresh single fibres from the semitendinosus muscle

(Hodgkin & Horowicz, 1959). The membrane potential is close to the theoretical line for $[K]_o > 10$ mM but deviates slightly at lower concentrations; possible reasons are considered in the next section.

In order to check that sulphate was acting as an impermeable ion, tests were also made with solutions in which there was no sulphate, and sodium chloride was replaced by an osmotically equivalent quantity of sucrose. The results, which are given in rows 10 and 15 of Table 2, agreed well with the Donnan theory.

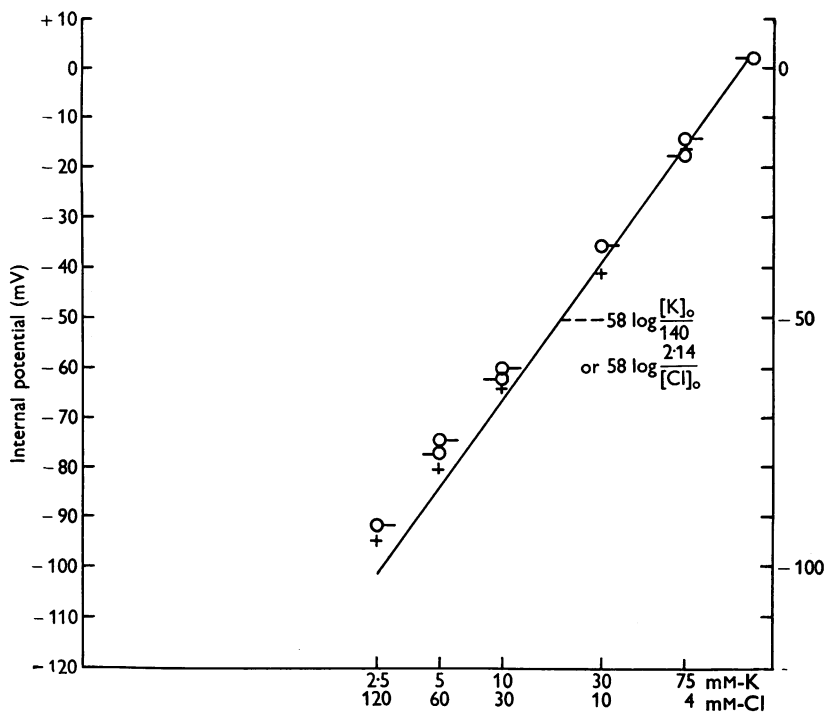


Fig. 4. Relation between membrane potential and $\log [K]_o$ or $-\log [Cl]_o$, when using solutions with $[K]_o [Cl]_o = 300 \text{ mM}^2$. Average data from two experiments, of which part of one is given in Fig. 3. Crosses are potentials measured after equilibrating for 10–60 min; circles are potentials measured 20–60 sec after a sudden change in concentration, \circ after increase in $[K]_o$, \circ after decrease in $[K]_o$. A correction for the change in activity coefficient has been made in plotting the right-hand point on the potassium scale; concentrations in this solution were 190 mM-K and 2.5 mM-Cl, the correction factor was 0.87.

One test was also made on a fibre which had been equilibrated with a solution containing 50 mM-K and 30 mM-Cl ($[K]_o [Cl]_o = 1500$); the results, which are given in Fig. 12, were also in good agreement with theory.

The action of solutions of constant K, Cl product on the membrane potential of the cat's gracilis muscle has recently been described by Pillat, Kraupp, Giebisch & Stormann (1958). These workers found a linear relation between

membrane potential and $\log [K]_o$ (or $\log [Cl]_o$) with a slope of 56 mV for a ten-fold change. Our results are clearly in good agreement with theirs and both are consistent with a Donnan theory of the type described by Boyle & Conway (1941).

Variation of $[K]_o$ in the absence of $[Cl]_o$

The effect of potassium on membrane potential can be studied most simply in the absence of chloride. This was done by Adrian (1956) who varied the potassium concentration between 10 and 190 mM. Adrian did not use lower concentrations because he found that muscles twitched spontaneously in sulphate solutions containing less than 10 mM-K. The object of the experiments described in this section was to see whether the membrane potential varied rapidly and reversibly with $[K]_o$ in the absence of Cl and, if possible, to extend the measurements to potassium concentration below 10 mM. The solutions, which were made by mixing solutions *D* and *E* (Table 1), contained more Ca and less SO_4 than those employed by Adrian; the ionic strength was the same as that of Ringer's fluid.

When a Cl-free solution containing 2.5 mM-K was first applied, the resting potential decreased by 30–40 mV and the fibre often twitched spontaneously. This depolarization was transient and after some time the resting potential in 2.5 mM-K sulphate was close to that in Ringer's fluid. An interval of 1 hr was usually allowed for equilibration, but this may be unnecessarily long. The fibres were electrically excitable and gave propagated action potentials and twitches. Later on it will be shown that the transient depolarization was probably caused by the fall in the e.m.f. of the chloride concentration cell, and that as chloride diffused from the fibre it ceased to affect the membrane potential, which then returned to the value determined by the potassium concentration.

Figure 5 illustrates the effect of $[K]_o$ on membrane potential in the absence of Cl; crosses are equilibrated values and circles are values established 20–60 sec after a sudden rise or fall of $[K]_o$. The range was extended beyond 80 mM-K by using an isotonic solution containing 190 mM-K (solution *H*, Table 1); the activity of potassium in this solution is estimated as $165 \times \gamma_R$ mM, where γ_R is the activity coefficient of potassium in Ringer's fluid. The potassium concentrations in the other solutions need no correction, since the ionic strength of the solutions was approximately the same as that in Ringer's fluid.

The membrane potential was established fairly rapidly (5–60 sec) and there was no slow drift or hysteresis of the kind associated with chloride movements. The potential varied between +2 mV in high K and about –120 mV in 0.5 mM-K. Reducing $[K]_o$ from 0.5 mM to zero caused the resting potential to fall by several millivolts; other examples of this type of behaviour are discussed on p. 147.

At high potassium concentrations the results agreed well with the Nernst

equation for a potassium electrode, as shown previously by Adrian (1956). The straight line in Fig. 5 is drawn from this equation at the same internal potassium concentration as in Fig. 4.

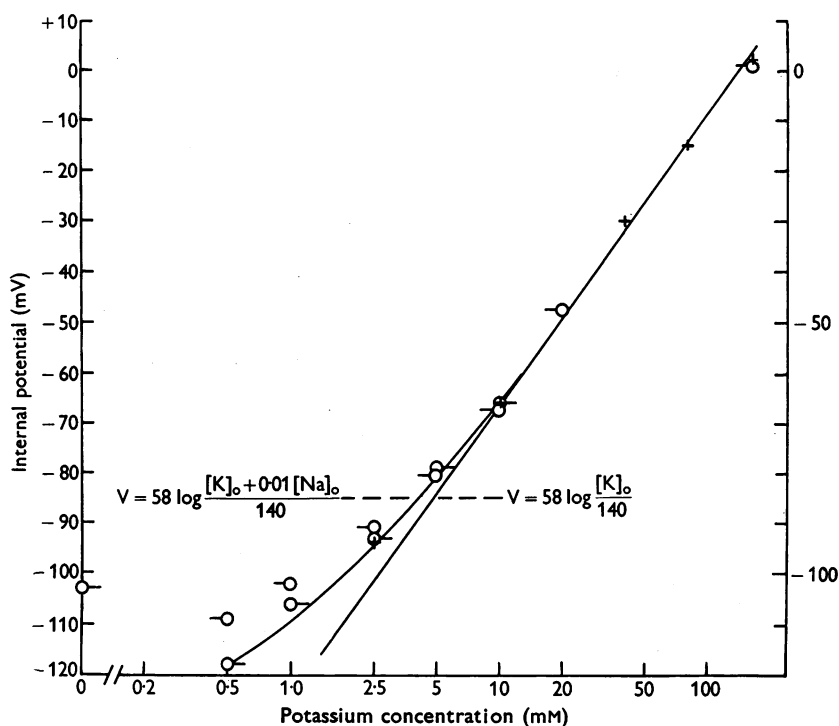


Fig. 5. Relation between membrane potential and $\log[K]$ when using chloride-free sulphate solutions containing 8 mM- CaSO_4 . Crosses are potentials measured after equilibrating for 10–60 min; circles are potentials measured 20–60 sec after a sudden change in concentration, \circ after increase in $[K]_o$, \bigcirc after decrease in $[K]_o$. With the exception of the right-hand point, all solutions were of the same ionic strength as Ringer's fluid and had concentrations intermediate between *D* and *E* of Table 1. The right-hand point was obtained with solution *H* and the potassium concentration has been corrected on the graph for the difference in ionic strength. Semi-log. scale; average data on seven fibres.

Between 10 and 0.5 mM the resting potential deviated from the line for a potassium electrode, the slope at 2.5 mM-K being about 40 mV. In this region the deviation is of the kind expected from a very slight permeability to some other ion, for example sodium. For concentrations greater than 1 mM the points are reasonably well fitted by a curve drawn according to the equation

$$V_{K+\alpha Na} = \frac{RT}{F} \ln \frac{[K]_o + \alpha [Na]_o}{[K]_i + \alpha [Na]_i}, \quad (4)$$

where α has the value of 0.01. Since $[K]_i$ is much greater than $[Na]_i$, $\alpha [Na]_i$ may be neglected.

An equation of this kind can be derived in various ways; for example, by the constant field theory of Goldman (1943). However, the relation is more general than the constant field theory because it follows from the initial postulate of Goldman's theory and does not require the somewhat arbitrary restriction of a constant field. If the definitions adopted by Hodgkin & Katz (1949) are employed, the constant α is equal to the permeability ratio P_{Na}/P_K .

Although the membrane potential in Cl-free solutions changed with $[K]_o$ in a reversible manner, the rate of repolarization when high K was removed was very much slower than the rate of depolarization when high K was applied. On the relatively slow time scale used in the present paper these changes may be regarded as instantaneous, but it will be shown in a subsequent article that the repolarization when high K is removed is too slow to be explained in terms of a straight-forward diffusion delay.

Membrane potentials in the steady state in different solutions

During the course of the experiments we accumulated many results on fibres which had been equilibrated in solutions of widely different composition;

TABLE 2. Membrane potentials after equilibration in different solutions

Row	K ⁺	Cl ⁻	Na ⁺	Choline ⁺	SO ₄ ²⁻	Sucrose	Membrane potential	V _{K+αNa}	V _K	No. of fibres
	(mm)						(mV)			
1	0.5	0	82	—	48	113	-109 to -118	-117	-142	1
2	0.5	120	122	—	—	—	-113	-111	-142	1
3	1.0	0	82	—	48	113	-103 to -113	-109	-124	3
4	1.0	120	5	117	—	—	-112	-123	-124	1
5	2.5	0	80	—	48	113	-94±1.0	-94	-101	8
6	2.5	120	120	—	—	—	-94±0.5	-91	-101	16
7	2.5	120	5	115	—	—	-100±1.4	-101	-101	11
8	5.0	0	78	—	48	113	-76 to -87	-80	-84	2
9	5.0	60	97	—	24	57	-76±2	-79	-84	2
10	5.0	60	56	—	—	118	-74 to -80	-81	-84	2
11	10	0	73	—	48	113	-68±0.9	-65	-66	5
12	10	120	113	—	—	—	-64±0.8	-64	-66	8
13	10	120	5	108	—	—	-61 to -63	-66	-66	2
14	10	30	83	—	36	85	-62±2	-65	-66	2
15	10	30	22	—	—	171	-61 to -63	-66	-66	1
16	30	10	55	—	44	103	-37±1.4	-38	-39	2
17	75	4	6	—	46	109	-16±1.4	-16	-16	2
18	80	6	3	—	48	113	-15±0.5	-14	-14	5
19	165	0	3	—	101	—	+1 to +2	+4	+4	2
20	165	2	3	—	101	—	+1 to +4	+4	+4	2
21	95	4	98	—	95	168	-21 to -23	-23	-23	3
22	95	214	120	—	—	—	-21±0.3	-23	-23	7

Rows 1 to 20 were obtained with solutions isotonic with Ringer's fluid, 21 and 22 with hypertonic solutions. The ionic strength was the same as in Ringer's fluid except in 10, 15, 19-22. Concentrations of K and Cl have been corrected for changes in activity coefficient in 10, 15, 19 and 20 but not in 21 and 22 since here the internal ionic strength is also increased. V_K is calculated from eqn. 2, $V_{K+\alpha Na}$ from eqn. 4 using $\alpha = 0.01$. $[K]_i$ is taken as 140 mm except in rows 21 and 22 where it is 235 mm. Between 2 and 6 observations were usually made on each fibre. Means and s.e. are given when the number of observations exceeds 6. Concentrations of Ca and phosphate are given in Table 1.

these are summarized in Table 2. Except in the last pair of measurements, fibres were equilibrated in solutions in which the internal potassium concentrations should remain close to its resting value of 140 mM (see Boyle & Conway, 1941; Adrian, 1956). The table shows that the membrane potential is the same in a solution containing a given amount of K and 120 mM-Cl as it is in the corresponding solution containing the same amount of K and zero Cl. The inference is either (i) that Cl^- does not contribute to the membrane current, which is unlikely since it is known to penetrate the fibre and since changes in Cl do in fact produce transient electrical changes or (ii) that chloride is distributed passively according to eqn. 1.

As may be seen from Table 2, the membrane potential agreed with that for a potassium electrode if $[\text{K}]_o > 10$ mM or with eqn. 4 if $[\text{K}]_o > 1$ mM.

In the last pair of measurements the tonicity of the external solution was increased 1.75 times by adding 93 mM-KCl, or an osmotically equivalent quantity of sucrose. According to the well-tried theory of Boyle & Conway, the internal potassium concentration should increase by 90–100 m-mole/kg H_2O under these conditions; a value of $[\text{K}]_i = 235$ m-mole/kg H_2O has therefore been employed. Potentials calculated on this basis agree with those found experimentally.

Variation of $[\text{Cl}]_o$ at constant $[\text{K}]_o$: experiments in the physiological range

Figure 6 illustrates the effect of suddenly reducing the concentration of chloride on the membrane potential of a fibre equilibrated in Ringer's fluid. The test solution (G, Table 1) contained sulphate and had the same osmotic pressure, ionic strength and potassium concentration as Ringer's fluid. When this solution was applied the resting potential fell from its resting value of -98.5 mV to -77 mV. However, this depolarization was not maintained and the potential drifted back to its original level with a time constant of about 4 min. On restoring Ringer's fluid the membrane potential first increased by 12 mV and then drifted back to its original value. Similar results were obtained in experiments in which chloride was reduced by replacing NaCl with an osmotically equivalent quantity of sucrose.

The transient effects in Fig. 6 are of the kind expected in a system in which the membrane is permeable to both K and Cl. Initially the e.m.f. of the chloride concentration cell should be equal to the resting potential; from eqn. 1 this requires that $[\text{Cl}]_i$ be 2.4 mM. On reducing the external chloride concentration from 120 to 30 mM the e.m.f. of the chloride concentration cell alters by 35 mV and V_{Cl} changes from -98.5 to -63.5 mV; the potassium equilibrium potential remains unchanged. The result of the reduction in $[\text{Cl}]_o$ is that the fibre is depolarized to a potential between V_{K} and V_{Cl} at which K^+ and Cl^- leave the fibre at the same rate. Loss of KCl continues until the

$[K]_i[Cl]_i$ product is reduced to one-fourth of its original value; at the same time water leaves the fibre in order to preserve osmotic equilibrium. From the theory of Boyle & Conway (1941) it can be shown that re-establishment of equilibrium involves loss of KCl and water in amounts such that $[Cl]_i$ is reduced to almost exactly 0.6 mM and $[K]_i$ is virtually unchanged; the final membrane potentials should therefore be the same in the two solutions. The transient hyperpolarization on returning to Ringer's fluid is explained in the same way. When Ringer's fluid is first applied $[Cl]_i$ is 0.6 m-mole/kg H_2O , so that V_{Cl} is about -134 mV as against about -100 mV for V_K . The membrane potential takes up an intermediate value—about -112 mV—at which the

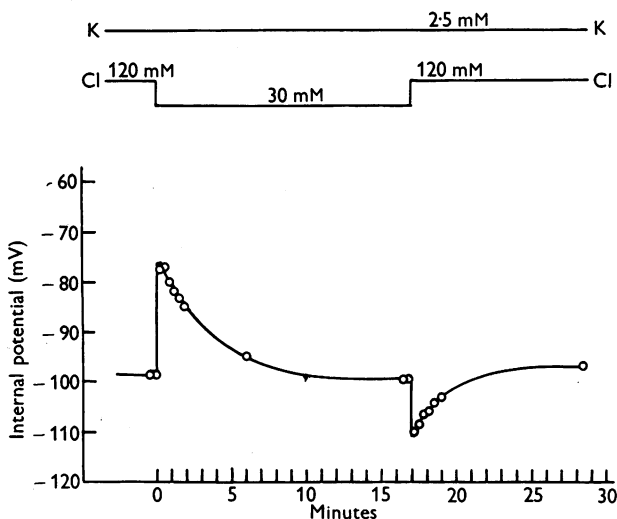


Fig. 6. Effect of sudden reduction in $[Cl]_o$ on membrane potential. The solutions were Ringer's fluid (A, Table 1) and an isotonic solution of the same ionic strength with reduced Cl (G, Table 1). The micro-electrode was kept in the fibre from -0.5 to 2 min and from 16 to 19 min; it was also inserted for about 0.3 min at 6 and 28 min. Control flushes with the solution already in the cell were made between the first and second points and between the two points immediately after the change at 17 min; these had no effect on the membrane potential. Fibre r , diameter 111μ , $23^\circ C$.

TABLE 3. Instantaneous effect of $[Cl]_o$ on fibres equilibrated in Ringer's fluid
(2.5 mM-K 120 mM-Cl)

$[K]_o$ (mM)	$[Cl]_o$ (mM)	Internal potential (mV)				Mean
		i	r_1	r_2	r_3	
2.5	120	-92.1	-93.4	-94.2	-98.5	-94.5
2.5	60	-80.0	-85.1	-84.1	—	-83.0
2.5	30	-64.5	-76.4	-75.2	-77	-72.0

Data from four runs on two fibres. Other experiments with slightly different solutions gave similar results but have been excluded for simplicity. In fibre r solutions with SO_4 of same ionic strength as Ringer's fluid were employed; in fibre i isotonicity was preserved with sucrose alone and concentrations have been corrected for the change in ionic strength. Fibres referred to specifically in this paper are denoted by letters; references such as r_1 or r_2 are used when there are several sets of measurements on one fibre.

rates of entry of K and Cl are equal. Entry of Cl then brings $[Cl]_i$ back to its normal value and the fibre is once more in equilibrium.

Chloride conductances and permeabilities can be calculated from the transient changes in Fig. 6 by the methods discussed on p. 150. For this particular experiment the chloride conductances found for the transient change on reducing Cl was $130 \mu\text{mho}/\text{cm}^2$ and that for the change on returning to Ringer's fluid was $90 \mu\text{mho}/\text{cm}^2$.

The data in Tables 3 and 6 summarize the instantaneous effect of changing $[Cl]_o$ on membrane potential in the physiological region. The average value of

$$\left(\frac{\partial V}{\partial \log [Cl]} \right)_{2.5 \text{ mM-K}}$$

is about -35 mV (see p. 149).

Giebisch, Kraupp, Pillat & Stormann (1957) studied the effect on the membrane potential of the cat's gracilis muscle of replacing part of the external NaCl with Na_2SO_4 or sucrose. In the former case they found a large depolarization, lasting about an hour, which they attributed to an effect of sulphate on sodium permeability (CaSO_4 was not added); in the latter, a small, maintained depolarization, which they explained in terms of the Donnan theory. A transient depolarization was not seen with sucrose but the measurements may not have been made sufficiently rapidly for this to appear.

Variation of $[K]_o$ at constant $[Cl]_o$: experiments in the physiological region

Figure 7 illustrates the effect of raising $[K]_o$ at constant $[Cl]_o$. The test solution contained 10 mM-K and 120 mM-Cl, instead of 2.5 mM-K and 120 mM-Cl as in Ringer's fluid. In *a*, 10 mM-K was applied for 8 sec, the resting potential fell from -94 to -73 mV and returned to -93 mV when 2.5 mM-K was restored; the half-times of these changes were 0.35 and 1.0 sec. In *b* the same solution was applied for 60 sec. The potential fell from -93 mV to -71 mV and returned to -87 mV . Curve *c*, which was obtained on another fibre, shows the effect of a long exposure. When 10 mM-K was first applied the internal potential changed rapidly from -94 to -73 mV and then drifted slowly to its equilibrium value of -65 mV . On restoring 2.5 mM-K, the initial repolarization was only about 3 mV and it took about 40 min to restore a resting potential of -90 mV . The asymmetry between the on- and off-effects of 10 mM-K was independent of the order in which the measurements were made. This was shown by experiments in which the fibre was first equilibrated in 10 mM-K 120 mM-Cl, then transferred to 2.5 mM-K 120 mM-Cl and finally returned to 10 mM-K 120 mM-Cl. The results were similar to those in Fig. 7*c* except that the order of the curves was reversed. An asymmetry of the kind shown in Fig. 7 has been seen in experiments with whole muscle by Sandow & Mandel (1951) and Csapo & Wilkie (1956).

The family of curves in Fig. 8 illustrates the instantaneous relation between $[K]_o$ and membrane potential for fibres equilibrated in solutions of different $[K][Cl]$ product. It will be seen that a rise of $[K]_o$ above the level used to

equilibrate gives a relatively large depolarization, whereas a fall of $[K]_o$ gives only a small hyperpolarization. A similar family of curves was obtained in solutions containing choline instead of Na^+ . When $[K]_o$ was reduced far below the level used to equilibrate, the resting potential did not reach a constant limiting value, as it seems to do in nerve, but passed through a flat maximum. For fibres equilibrated in Ringer's fluid the maximum potential was established between 0.5 and 1.0 mM-K and the resting potential was 3 mV less in 0 mM-K than in 0.5 mM-K. An exaggerated example of this type of behaviour is discussed on p. 147.

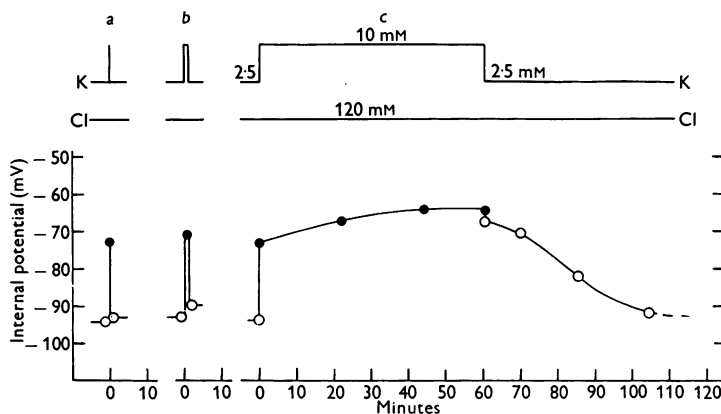


Fig. 7. Effect on membrane potential of changing $[K]_o$ from 2.5 to 10 mM at constant Cl. O, observations with 2.5 mM-K, ●, observations with 10 mM-K; the two solutions were A (Ringer's fluid) and B of Table 1. Results a and b were obtained on fibre *l* diameter 119μ and c on fibre *m*, diameter 173μ . In a and b the micro-electrode was left inside the fibre; in c it was inserted for about 0.5 min at a time. Temperature $20^\circ C$. Some time after the last observation in c the region of the fibre which had been impaled became opaque and the resting potential underwent a gradual decline.

The effects illustrated by Figs. 7 and 8 can be explained by assuming that (1) both K^+ and Cl^- cross the membrane and that at equilibrium the distributions of these ions conforms with the Donnan principle; and (2) the potassium system can pass a large current in high K if the driving force is inward ($V < V_K$) but only a small current if the driving force is outward ($V > V_K$). The second assumption agrees with the observation of Katz (1949) who showed that muscles in isotonic potassium sulphate solution have a low membrane resistance for inward current but a high resistance for outward current. On this basis the effect of raising K at constant Cl (Fig. 7) can be explained in the following way. When $[K]_o$ is changed from 2.5 to 10 mM, V_K alters by 35 mV; this causes inward current to flow through the potassium channel and V approaches V_K . The membrane potential is now between V_K and V_{Cl} so that KCl (and water) enter the fibre until equilibrium is established with

$$V = V_K = V_{Cl} = -65 \text{ mV.}$$

On returning to 2.5 mM-K, V_K at once reverts to its original value of about 100 mV. Since the potassium current is outward the potassium resistance is high, so that the fibre at first repolarizes by only 2 or 3 mV. However, the existence of this small change means that the fibre is no longer in equilibrium with respect to chloride ions and KCl slowly leaves the fibre until the original equilibrium is restored.

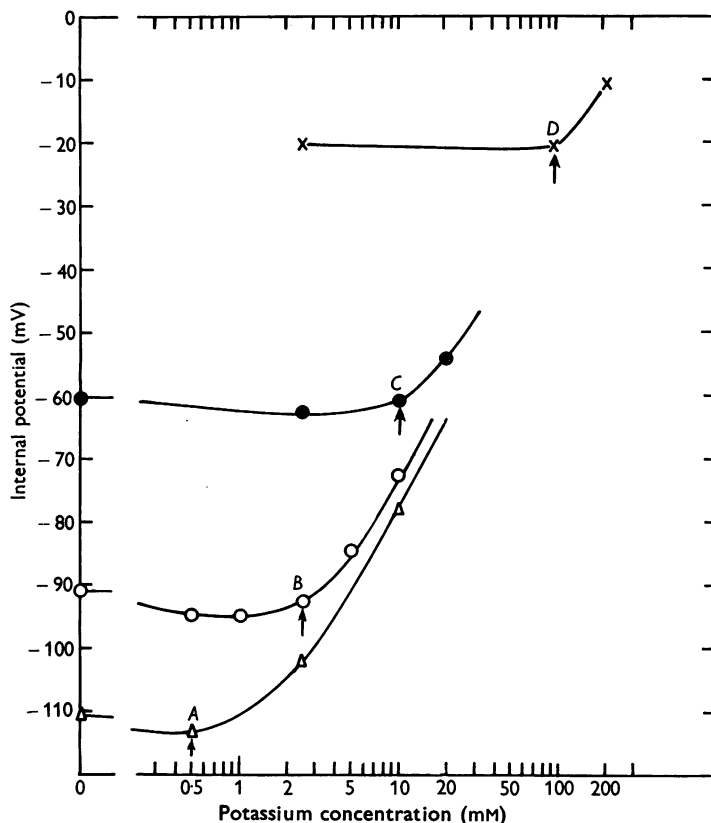


Fig. 8. Instantaneous effect of varying $[K]_o$ at constant $[Cl]_o$ on membrane potential of fibres equilibrated in A, 0.5 mM-K 120 mM-Cl; B, 2.5 mM-K 120 mM-Cl (Ringer's fluid); C, 10 mM-K 120 mM-Cl; D, 95 mM-K 214 mM-Cl. The arrows show the potassium concentration at which the fibre was equilibrated. The shape of curve D is doubtful. Curve A, fibre *b*; curve B, mean from four fibres; curve C, mean from three fibres; curve D, fibre *p*. In A, B and C, $[Na]_o + [K]_o = 122.5$ mM; in D, $[Na]_o + [K]_o = 215$ mM. Semi-log. scale.

Variations of $[K]_o$ or $[Cl]_o$; fibres equilibrated in solutions of high $[K] [Cl]$ product

The internal potential of fibres equilibrated in a solution containing 95 mM-K 214 mM-Cl was -20.6 ± 0.3 mV (mean and s.e., 11 observations). When $[K]_o$ was increased at constant $[Cl]_o$ to 210 mM the fibre was depolarized

to -11 mV, but if $[K]_o$ was reduced to 2.5 mM the change in potential was less than 0.5 mV (Fig. 8, top curve). Since there was no sudden change in potential on reducing $[K]_o$ to 2.5 mM and since the internal chloride concentration was probably about 90 mM, one would expect any recovery of resting potential to be exceedingly slow. This is one of the points illustrated by Fig. 9. It will be shown presently that failure to repolarize is not the result of irreversible damage, since a normal resting potential can be restored if the internal chloride is first lowered by immersing the fibre in a chloride-deficient solution.

According to the hypothesis outlined on p. 140, the insensitivity to a reduction of $[K]_o$ is caused by the rise in internal chloride concentration and by rectification in the potassium channel. If the argument is correct, the membrane should become sensitive to a reduction of $[Cl]_o$ as it loses its ability to repolarize in low $[K]_o$. Figure 9 shows that the ability to repolarize in 2.5 mM-K disappeared during the first 50 min of the immersion in the solution of high K, Cl product. Figure 10, which was obtained on another fibre, shows that during this period the fibre became sensitive to a reduction of $[Cl]_o$. When the fibre had reached equilibrium in 95 mM-K 214 mM-Cl, reducing the chloride concentration to 3.6 mM made the internal potential swing to $+64$ mV. In common with other changes produced by Cl this effect was transient, and after about 1 hr the potential returned to the potassium equilibrium potential of -20 mV. On replacing Ringer's fluid a resting potential of -88 mV was obtained. Recovery was less complete in the experiment of Fig. 9 but this may have been the result of injury in the impaled region. Some fibres gave propagated twitches at the end of the experiment, others which showed signs of damage in the impaled region did not. Considering the drastic treatment and the number of impalements, we regard the recovery in Fig. 10 as satisfactory evidence that the internal chloride concentration can be raised to a high level without causing gross, irreversible damage to the membrane.

Table 4 and curve *A* in Fig. 11 give the relation between $[Cl]_o$ and internal potential on fibres which have been equilibrated in 95 mM-K 214 mM-Cl. With chloride concentrations down to 30 mM, the membrane behaved like a chloride electrode, the slope for a tenfold change of chloride concentration being 56 mV (55 mV for a tenfold change of activity). As a corollary, it was found that the potential was little affected by changing from K to Na at a fixed chloride concentration (Table 4, fibre *f*).

The curve drawn through the points in Fig. 11 (curve *A*) has a slope of about 45 mV between 214 and 107 mM-Cl and 65 mV between 107 and 30 mM-Cl. This is probably not the result of experimental error, since a curve of this shape is explained by a decrease of potassium permeability as the internal potential becomes more positive. With 2.5 mM-K instead of 95 mM-K, the relation between *V* and log Cl should, and probably does, approximate more closely to that of a perfect chloride electrode (Table 4, fibre *f*2).

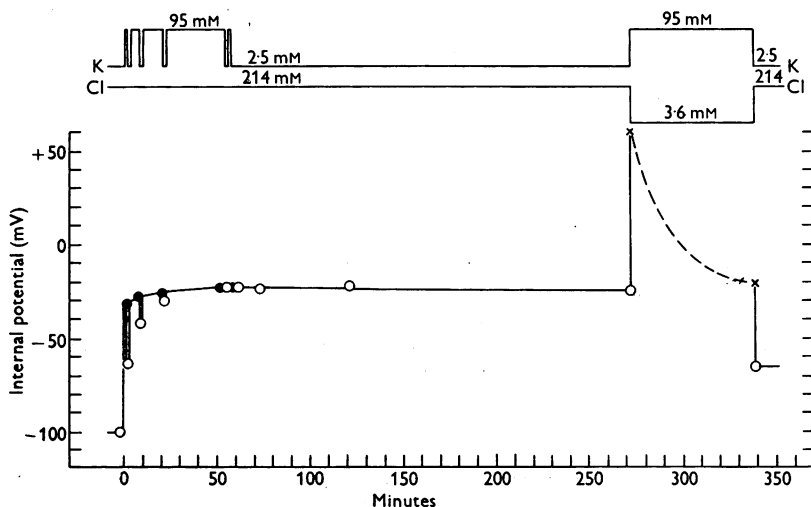


Fig. 9. Effect of large changes in $[K]_0$ and $[Cl]_0$ on membrane potential. The tonicity of the three solutions used was 1.75 times that of Ringer's fluid. \circ , 2.5 mM-K 214 mM-Cl; \bullet , 95 mM-K 214 mM-Cl; \times , 95 mM-K 3.6 mM-Cl. Fibre *j*; diameter $132\ \mu$, temperature 20°C .

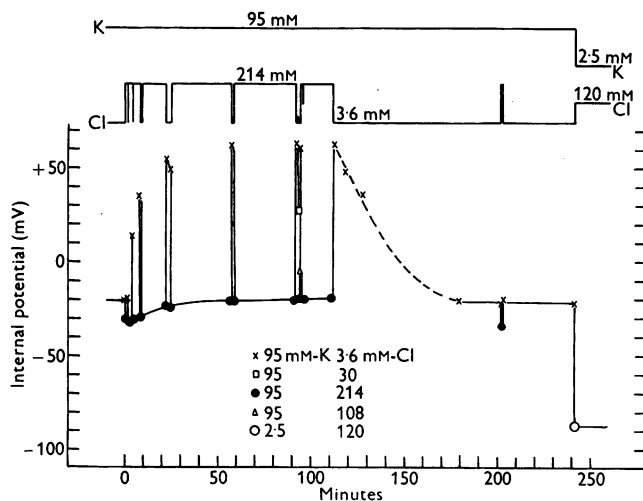


Fig. 10. Effect of large changes in $[K]_0$ and $[Cl]_0$ on membrane potential. All the observations except the final measurement in Ringer's fluid (2.5 mM-K 120 mM-Cl) were made with solutions isotonic with 2.5 mM-K 214 mM-Cl. Fibre *e*, $133\ \mu$, temperature 21°C . The main point illustrated by the figure is that as the fibre became loaded with chloride in 95 mM-K 214 mM-Cl the internal potential in 95 mM-K 3.6 mM-Cl changed from -21 mV to $+63\text{ mV}$; on leaving the fibre in the latter solution the potential eventually returned to -21 mV and the fibre then repolarized to -88 mV in Ringer's fluid (2.5 mM-K 120 mM-Cl).

TABLE 4. Instantaneous effect of $[Cl]_o$ on fibres equilibrated in 95 mM-K 214 mM-Cl

$[Cl]_o$ (mm)	Internal potential (mV)									Mean	$V_{calc.}$
	e_1	e_2	e_3	f_1	f_2	o	p_1	p_2	q		
214	-20.9	-20.0	-19.2	-19	-18.5	-22.3	-20.2	-20.2	-20.4	-20.3	(-20.5)
107	—	-4.7	—	—	-1.7	-8.6	—	—	—	-6.7	-3.1
30	—	+27.5	—	—	+29.2	+25.2	+27.3	+27.6	+28.9	+27.3	+29.0
3.6	+62.3	+63.1	+62.6	+73	+74	—	—	—	—	+65.3	+82.0
3.0	—	—	—	—	—	+71.4	—	—	—	+71.4	+87.0
0	—	—	—	—	—	+84.5	—	—	—	+84.5	$+\infty$

All the test solutions were isotonic with the solution used for equilibration (95 mM-K 214 mM-Cl). In f_2 the test solutions contained 2.5 mM-K and 212.5 mM-Na; in the other experiments they contained 95 mM-K and 120 mM-Na. f_2 is excluded from the mean. $V_{calc.}$ is $58 \log 95/[Cl]_o$. The figure of 95 is the value of $[Cl]_i$ which gives a potential of -20.5 mV in row 1; it is also the value predicted by the Donnan theory if the indiffusible 'anions' are monovalent (see Boyle & Conway, 1941).

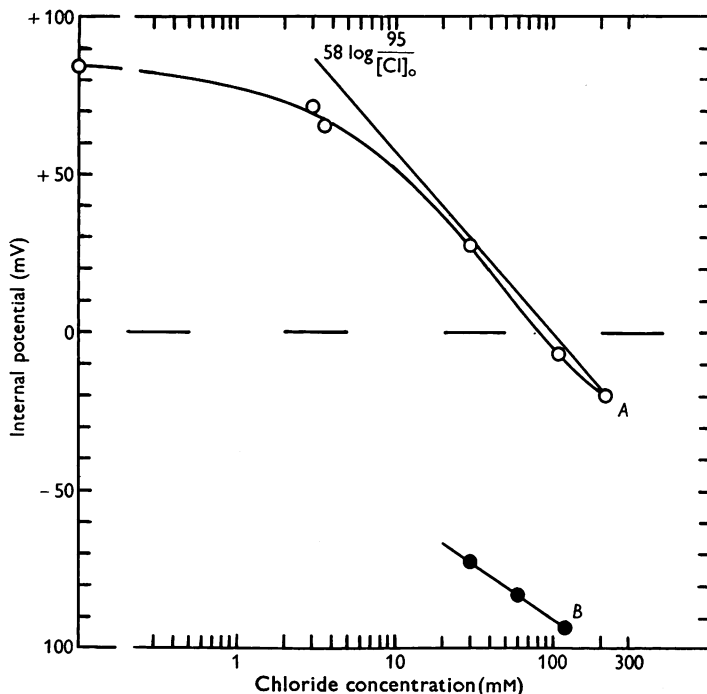


Fig. 11. Instantaneous relation between $[Cl]_o$ and membrane potential using fibres equilibrated in A, 95 mM-K 214 mM-Cl; B, 2.5 mM-K 120 mM-Cl (Ringer's fluid). A, data from Table 4, all solutions contained 95 mM-K and were isotonic with 95 mM-K 214 mM-Cl. B, data from Table 3, excluding the last set of measurements which are incomplete; all solutions contained 2.5 mM-K and were isotonic with Ringer's fluid. Semi-log. scale.

At chloride concentrations below about 30 mM the curve flattens, reaching a limiting value of about +80 mV in chloride-free solutions. This high value was not maintained for more than a few seconds and it seems possible that the maximum internal potential was limited by a break-down of the membrane. However, this was not the only explanation of the flattening, since a departure from V_{Cl} at low $[Cl]_o$ was also seen at smaller potentials when the fibre had been equilibrated with solutions which raised the internal chloride to a less extent. Other explanations are that the permeability to potassium is not completely shut off or that the permeability to sulphate may not be zero. In terms of the constant field theory (Goldman, 1943), the permeability ratios P_K/P_{Cl} or P_{SO_4}/P_{Cl} need to be about 0.01 in order to explain the departure from the Nernst relation at low $[Cl]_o$.

Effect of alterations in the internal chloride concentration on the membrane potential; fibres equilibrated in 95 mM-K, 214 mM-Cl

Using sartorius muscles in Cl-free sulphate, Adrian (1956) showed that an increase of external osmotic pressure at constant $[K]_o$ made the inside of the fibre more negative by about the amount calculated from the increase in the internal potassium concentration. When a muscle fibre is in a condition where the membrane potential is dominated by Cl^- , rather than by K^+ as in Adrian's experiments, an increase in osmotic pressure ought to have the opposite effect.

TABLE 5. Effect of changing tonicity on membrane potential of fibres loaded with Cl

Solution	$[K]_o$	$[Cl]_o$	$[Cl]_i$ calc.	Sucrose	Relative tonicity	Internal potential				
						P_1	P_2	q (mV)	Mean	$V_{calc.}$
1	95	214	95	0	1.75	-20.0	-20.0	-20.9	-20.3	(-20.5)
2	95	30	95	147	1.75	+27.2	+27.6	+28.9	+27.9	+29.0
3	95	30	60	0	1.10	+16.7	+15.2	+16.0	+16.0	+17.4
2	95	30	95	147	1.75	+27.4	+27.6	+28.8	+27.9	+29.0
1	95	214	95	0	1.75	-20.3	-20.4	-19.8	-20.2	-20.5

Solution 1 was solution *J* in Table 1. Solution 2 was intermediate between *J* and *K* in Table 1. Solution 3 was identical with 2 except for the absence of sucrose. The tonicity is given relative to that of Ringer's fluid. The fibres were equilibrated in 95 mM-K 214 mM-Cl for about 100 min. The first application of solution 2 lasted about 30 sec but the subsequent applications of 3 and 2 lasted 40–90 sec. The above measurements were made from the records when the potential had reached a steady value. $V_{calc.}$ is $58 \log \frac{[Cl]_i}{[Cl]_o}$ with $[Cl]_i = 95$ mM for solutions 1 and 2 or 60 mM for solution 3. Note that $\frac{60}{95} = \frac{1.10}{1.75}$.

This is borne out by the experiments shown in Table 5. It will be seen that the change in membrane potential is close to that calculated for a chloride electrode on the assumption that the fibre attains osmotic equilibrium. The effects of added sucrose were fully reversible and took place with a half-time of 6–12 sec. The water permeability calculated from these records was about

10 μ /min atm, i.e. about 3 times greater than in mammalian red cells and 100 times greater than in *Arbacia* eggs (see Lucké & McCutcheon, 1932). Details of the analysis will be given elsewhere.

Variations of $[K]_o$ and $[Cl]_o$; fibres equilibrated in solutions of intermediate K, Cl product

When a fibre has been equilibrated in a solution of high K, Cl product (e.g. 95 mM-K 214 mM-Cl), reduction of $[K]_o$ to a low value produces virtually no change in membrane potential. Under these conditions the chloride conductance is so much larger than the potassium conductance that chloride ions dominate the membrane potential. Interesting information was obtained by equilibrating with solutions of moderate K, Cl product, 50 mM-K 30 mM-Cl being found convenient. These results are given in Fig. 12. It will be seen from curve I that reducing $[K]_o$ from 50 to 25 mm (at constant $[Cl]_o$) caused the fibre to repolarize from -32 to -38 mV, but that a further reduction (again at constant $[Cl]_o$) from 25 to 2.5 mM-K returned the potential to -32 mV. This effect was fully reversible. On going straight from 50 into 2.5 mM or from

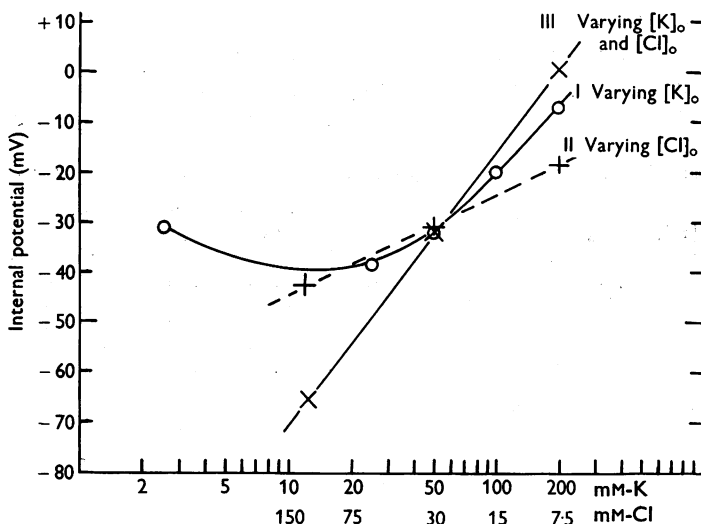


Fig. 12. Effect of varying $[K]_o$ and $[Cl]_o$ on fibre equilibrated in solution containing 50 mM-K 30 mM-Cl.

Curve I (\circ), variation of $[K]_o$ at constant $[Cl]_o$;

Curve II (+), variation of $[Cl]_o$ at constant $[K]_o$;

Curve III (\times), simultaneous variation of $[K]_o$ and $[Cl]_o$ with $[K]_o [Cl]_o = 1500 \text{ (mm)}^2$.

The curves were done in the order II, I, III; incomplete measurements indicated that the slope of curve I at 50 mM-K was about 10% steeper at the beginning of the experiment. The solution used for equilibration consisted of (mM) K 50, Cl 30, Na 152, Ca 8, SO_4 93, phosphate 1.5. The tonicity of all solutions was about 1.13 times that of Ringer's fluid except for the left-hand point of curve III, in which the solution had the same tonicity as Ringer's fluid. Fibre *n*; diameter, 134 μ , temperature, 22° C. Semi-log. scale.

2.5 to 50 mm there was no permanent displacement of membrane potential, but in both cases there was a transient repolarization lasting 5–10 sec; this effect, which will be discussed in a subsequent paper, suggests that the effective potassium concentration at the membrane varies more slowly than the concentration in the external solution. The probable explanation of the fall in resting potential at low $[K]_o$ is that the potassium permeability depends on external $[K]_o$ and that, at a fixed membrane potential, the permeability is greater when $[K]_o$ is high than when it is low. The result is that as $[K]_o$ is reduced the resting potential should pass through a maximum (minimum internal potential) and should then decline as the fall in potassium permeability outweighs the increase in the e.m.f. of the potassium concentration cell.

A decline in resting potential at low $[K]_o$ was also seen in Fig. 8 (variation of $[K]_o$ at 120 mm-Cl) and in Fig. 4 (variation of $[K]_o$ in the absence of Cl). In the former case the membrane potential should return to V_{Cl} if the potassium permeability fell to zero; in the latter, one must suppose that when $[K]_o$ is reduced to a very low value some other ion tends to control the potential. The other ion might be Cl^- which had diffused from the micro-electrode, Na^+ in the external medium, or a metabolic product such as HCO_3^- or lactate.

Line II in Fig. 12 gives the effect of varying Cl at constant K; line III was obtained by varying both K and Cl, keeping the KCl product constant. These results are considered further on p. 149.

The relative magnitudes of the K and Cl conductances under equilibrium conditions

The results described so far indicate that both K and Cl affect the membrane potential and that, under appropriate conditions, the membrane can be made to behave either as a potassium electrode or as a chloride electrode. In the remaining sections of this paper, similar results will be used to calculate the conductances and permeabilities of the membrane to the potassium and chloride ions. The underlying assumption, which receives further support from the experiments in this section, is that the K and Cl concentration cells are the main sources of membrane current and that the two cells are arranged in parallel, as shown in Fig. 13. Before considering absolute magnitudes we shall describe a method of estimating the ratio of the potassium and chloride conductances under equilibrium conditions. This method depends on the following argument.

The conductances are defined by

$$I_K = g_K(V - V_K), \quad (5)$$

$$I_{Cl} = g_{Cl}(V - V_{Cl}), \quad (6)$$

where I_K or I_{Cl} are the potassium or chloride current densities in the outward

direction and g_K or g_{Cl} are the corresponding conductances per unit area. Neither conductance will be assumed constant and both may vary in an arbitrary manner with concentration or potential. To begin with, it is assumed that only K^+ and Cl^- carry current through the membrane. In the absence of external current $I_K + I_{Cl} = 0$ and

$$V = V_K T_K + V_{Cl} T_{Cl}, \quad (7)$$

where

$$T_K = \frac{g_K}{g_K + g_{Cl}}. \quad (8)$$

and

$$T_{Cl} = 1 - T_K = \frac{g_{Cl}}{g_K + g_{Cl}}. \quad (9)$$

From (7) and (9) we obtain

$$\left(\frac{\partial V}{\partial V_K} \right)_{V_{Cl}} = T_K + (V_K - V_{Cl}) \left(\frac{\partial T_K}{\partial V_K} \right)_{V_{Cl}}, \quad (10)$$

and

$$\left(\frac{\partial V}{\partial V_{Cl}} \right)_{V_K} = T_{Cl} + (V_{Cl} - V_K) \left(\frac{\partial T_{Cl}}{\partial V_{Cl}} \right)_{V_K}. \quad (11)$$

For a small change from an equilibrium state in which $V_K = V_{Cl}$ these relations become

$$\left(\frac{\partial V}{\partial V_K} \right)_{V_{Cl}} = T_K, \quad (12)$$

$$\left(\frac{\partial V}{\partial V_{Cl}} \right)_{V_K} = T_{Cl}. \quad (13)$$

Hence

$$\left(\frac{\partial V}{\partial V_K} \right)_{V_{Cl}} + \left(\frac{\partial V}{\partial V_{Cl}} \right)_{V_K} = 1. \quad (14)$$

If V_K and V_{Cl} are constant and V is varied, the fraction of membrane current carried by K^+ is T_K and by Cl^- is T_{Cl} ; these quantities are therefore the transport numbers of K and Cl .

For comparison with experiments it is convenient to express V in millivolts and, using eqns. 2 and 3, to rewrite eqns. 12-14 in the form

$$\left(\frac{\partial V}{\partial \log [K]_o} \right)_{[Cl]_o} = 58 T_K, \quad (15)$$

$$- \left(\frac{\partial V}{\partial \log [Cl]_o} \right)_{[K]_o} = 58 T_{Cl}, \quad (16)$$

and

$$\left(\frac{\partial V}{\partial \log [K]_o} \right)_{[Cl]_o} - \left(\frac{\partial V}{\partial \log [Cl]_o} \right)_{[K]_o} = 58. \quad (17)$$

A more exact formula which takes account of the imperfect selectivity of the membrane (eqn. 4) is obtained by substituting $([K]_o + 0.01[Na]_o)$ for $[K]_o$ in eqn. 17.

Table 6 gives the results of experiments designed to test eqn. 17 and to estimate T_K and T_{Cl} . The quantities $\partial V/\partial \log[K]_o$ and $\partial V/\partial \log[Cl]_o$ were obtained from curves such as those in Fig. 8 or Fig. 12 by drawing a tangent at the concentration with which the fibre had been equilibrated. In order to reduce errors from progressive changes, the curve obtained by varying one ion (e.g. K) was bracketed with two curves obtained by varying the other (e.g. Cl).

TABLE 6. Slopes of curves relating potential to log. concentration

I Fibre reference	II [K] _o (mM)	III [Cl] _o (mM)	IV V (mV)	V $\left(\frac{\partial V}{\partial \log[K]_o}\right)_{Cl_o}$ (mV)	VI $-\left(\frac{\partial V}{\partial \log[Cl]_o}\right)_{K_o}$ (mV)	VII Sum of V + VI (mV)	VIII T_K	IX T_{Cl}
<i>i</i>	2.5	120	-94	14	40	54	0.24	0.69
<i>r</i>	2.5	120	-95	18	30	48	0.31	0.52
<i>n</i>	50	30	-32	36	19	55	0.62	0.33

Columns II and III give the concentrations used to equilibrate and IV the potential at equilibrium. V and VI give the slopes at the equilibrium point. VII is

$$\left(\frac{\partial V}{\partial \log[K]_o}\right)_{Cl_o} - \left(\frac{\partial V}{\partial \log[Cl]_o}\right)_{K_o}.$$

In *i* and *n* the measurements were made by varying first K, then Cl and finally K again; in *r* the order was reversed. T_K and T_{Cl} are defined in the text. The Cl curve and the second K curve for fibre *n* are shown in Fig. 12.

It will be seen that there is reasonable agreement with eqn. 17 and that, for the fibres equilibrated in Ringer's fluid, the transport numbers are approximately $T_K = 0.3$ and $T_{Cl} = 0.6$. If instead of the quantity

$$\frac{\partial V}{\partial \log[K]_o} \quad \text{we use} \quad \frac{\partial V}{\partial \log([K]_o + 0.01[Na]_o)}$$

the figures in column V become 21, 27 and 37 mV; on adding these values to the figures for $\partial V/\partial \log[Cl]_o$, the results are 61, 57 and 56 mV as against the theoretical value of 58 mV.

In two of the experiments in Table 6 we also measured the changes in potential produced by varying both K and Cl at constant product. This did not provide any new information but was a good way of checking the previous measurements since the line obtained by varying $\log[K]_o$ and $-\log[Cl]_o$ simultaneously ought to have the same slope as that obtained by making the measurements separately and adding the results. The slopes found were 53 mV for the first experiment and 56 mV for the third experiment, as against 54 and 55 mV in column VII of Table 6.

Absolute magnitude of g_{Cl} and P_{Cl}

There are several ways of estimating the chloride conductance, g_{Cl} , from the results described in this paper. Although differing in detail, the methods used all depend on calculating the internal chloride concentration from the external concentration and the membrane potential.

A. This method will be described in terms of the experiment illustrated by Fig. 9. At the beginning of the experiment the membrane potential in 2.5 mM-K 214 mM-Cl was -100.5 mV. On substituting this value in eqn. 3, $[Cl]_i$ is found to be 3.96 m-mole/kg H_2O . When 95 mM-K 214 mM-Cl was applied the potential changed to -31.7 mV. After 104 sec in this solution, reduction of $[K]_o$ to 2.5 mM caused the membrane potential to change to -63.9 mV, which is taken to be the new value of V_{Cl} ; from eqn. 3 $[Cl]_i$ is therefore 16.9 m-mole/kg H_2O . From the rate of rise of chloride concentration, the fibre diameter and the water content of muscle, the inward flow of chloride is found to be 333 pmole/cm² sec. The flow occurs from a concentration of 214 mM into one of 10.4 m-mole/kg H_2O against a potential difference of 31.7 mV (10.4 is the mean of 3.96 + 16.9). The chloride conductance, g_{Cl} , is therefore

$$\frac{333 \times 10^{-12} \times 96500}{(58 \log 214/10.4 - 31.7) \times 10^{-3}} = 720 \mu\text{mho/cm}^2.$$

The same method was applied to the results obtained when $[K]_o$ was raised for a short time from 2.5 to 10 mM at constant $[Cl]_o$, for example Fig. 7b.

B. This was similar to A but used the results given in Fig. 10. If V_t is the potential in 3.6 mM-Cl after t min in 95 mM-K 214 mM-Cl and V_∞ is the potential in 3.6 mM-Cl after a long time in 95 mM-K 214 mM-Cl, $[Cl]_t$, is taken as

$$[Cl]_t = [Cl]_\infty \exp \frac{F(V_t - V_\infty)}{RT}.$$

This equation follows from the assumption that the membrane behaves like a chloride electrode with respect to changes in internal chloride concentration (for evidence see Table 5).

C. When a fibre which has been equilibrated in 10 mM-K 120 mM-Cl is suddenly treated with 2.5 mM-K 120 mM-Cl, the potential at first changes from -65 to -68 mV and then slowly drifts towards -95 mV (Fig. 7c). Using eqn. 3 $[Cl]_i$ can be obtained from the first value, -65 mV, and $d[Cl]_i/dt$ can be estimated from the initial rate of repolarization. From the flow of Cl^- and the driving force (3 mV), g_{Cl} can be calculated in the usual way. This method was liable to introduce large errors, since neither the driving force nor the rate of repolarization could be measured accurately.

D. The fourth method depended on measuring the rate of change of membrane potential following a sudden change in $[Cl]_o$. For example in Fig. 6 when $[Cl]_o$ was suddenly reduced from 120 to 30 mM, the potential changed from -99 to -77 mV and then drifted back to its original value with a time constant whose initial value was 220 sec. The change in $[Cl]_i$ was from 2.4 to 0.6 m-mole/kg H_2O (p. 138) so the initial rate of fall of $[Cl]_i$ was 1.8/220 m-mole/kg H_2O per second. The driving force, $V - V_{Cl}$, is taken as the difference between the observed change in potential, 22 mV, and that expected for a chloride electrode, 34.9 mV.

Table 7 gives the results of these calculations. In the physiological region the chloride conductance is about $190 \mu\text{mho/cm}^2$ but higher values were obtained in fibres depolarized by 95 mM-K 214 mM-Cl. Since the conductance is likely to vary with $[Cl]_o$ and $[Cl]_i$ and with membrane potential it is desirable to calculate some other quantity as a measure of the permeability of the membrane to chloride. For this purpose it is convenient to use the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949) and to calculate a permeability coefficient, P_{Cl} , from the equation

$$P_{Cl} = M_{Cl} \frac{RT}{VF} \frac{1 - \exp(-VF/RT)}{[Cl]_o - [Cl]_i \exp(-VF/RT)}, \quad (18)$$

where M_{Cl} ($= I_{Cl}/F$) is the net flow of chloride into the muscle fibre. Table 7 shows that P_{Cl} is more constant than g_{Cl} and the apparent variation in P_{Cl} may be caused by experimental errors or by differences between fibres.

The conclusion from Table 7 is that movements of chloride occur in a relatively straight-forward manner and that the permeability to chloride is little influenced by changes in potential or concentration. The only observations which seem inconsistent are those made on fibres which have acquired a large and positive internal potential as a result of being transferred to low $[Cl]_o$ after equilibration in a solution of high $[K][Cl]$ product. In Fig. 10 the potential in low $[Cl]_o$ declines from its initial value of +60 mV at a rate of about 2 mV/min. If this decline is attributed solely to loss of KCl from the fibre, P_{Cl} is found to have a value of roughly 50×10^{-6} cm/sec instead of $3-5 \times 10^{-6}$ cm/sec as in other cases. The most likely explanation is that the

TABLE 7. Chloride conductances and permeabilities

Row	Fibre reference	Method	Fibre diameter (μ)	$[Cl]_o$ (m-mole/kg H ₂ O)	$[Cl]_i$ (m-mole/kg H ₂ O)	V (mV)	V_{Cl} (mV)	$V - V_{Cl}$ (mV)	Flow of Cl	g_{Cl} (μ mho/cm ²)	P_{Cl} (10^{-6} cm/sec)
									Inward = + Outward = - (pmole/cm ² sec)		
1	j_1	A	132	214	10.4	- 31.7	- 76.2	+ 44.5	+ 333	720	3.8
2	j_2	A	132	214	28.2	- 29.6	- 51.1	+ 21.5	+ 165	740	2.6
3	j_3	A	132	214	52.5	- 27.0	- 35.4	+ 8.4	+ 97	1100	2.9
4	e	B	133	214	22.4	- 31.0	- 56.9	+ 25.9	+ 253	940	3.9
Mean										875	3.3
5	c	A	87	120	4.49	- 66.8	- 82.8	+ 16.0	+ 37.2	220	3.3
6	d	A	118	120	5.13	- 69.0	- 79.4	+ 10.4	+ 50.6	470	6.6
7	a_1	A	148	120	2.79	- 71.5	- 94.7	+ 23.2	+ 32.6	(130)	(2.6)
8	l	A	119	120	3.37	- 72.3	- 90.0	+ 17.7	+ 46.6	250	4.5
9	h_1	D	84	28	2.88	- 70.4	- 57.3	- 13.1	- 14.3	105	4.1
10	h_2	D	84	28	3.59	- 62.3	- 51.7	- 10.6	- 9.15	83	2.7
11	a_2	C	148	120	11.52	- 62.4	- 59.0	- 3.4	- 11.7	(330)	(3.0)
12	m	C	173	120	9.28	- 67.6	- 64.5	- 3.1	- 5.8	180	1.9
13	r_1	D	111	30	2.40	- 77.0	- 63.6	- 13.4	- 18.5	130	5.8
14	r_2	D	111	120	0.60	- 111.0	- 133.5	+ 23.5	+ 22.2	90	5.5
Mean, second group										191	4.3
Mean, all measurements											4.0

Temperature 19–23°C. For $[K]_o$ in solutions, see Table. 8. Fibre a was in choline Ringer's fluid and has been omitted from the averages. P_{Cl} was calculated by eqn. 18.

membrane does not maintain its selective properties when subjected to a large reversed potential difference for long periods. On this view, the initial rapid disappearance of the positive internal potential is due not so much to loss of Cl as to the gradual development of an indiscriminate leak through the membrane.

The chloride conductance of a fibre equilibrated in Ringer's fluid can be calculated from the average value of P_{Cl} . When V is close to V_{Cl} it follows from eqns. 6 and 18 that the two units are related by

$$g_{Cl} = P_{Cl} \frac{F^3 V}{(RT)^2} \frac{[Cl]_o [Cl]_i}{[Cl]_i - [Cl]_o} \quad (19)$$

Taking P_{Cl} as 4×10^{-6} cm/sec, V as -95 mV, $[Cl]_o$ as 120 mM and $[Cl]_i$ as 2.8 m-mole/kg H₂O, g_{Cl} is found to be 170μ mho/cm². A similar value is obtained by averaging the observed values of g_{Cl} in the lower part of Table 7.

However, since g_{Cl} varies with concentration and potential it is better to use a value calculated from P_{Cl} . On p. 149 it was shown that in a fibre equilibrated in Ringer's fluid the potassium conductance was approximately half the chloride conductance. From this it follows that the total membrane conductance $g_K + g_{Cl}$ should be $1.5 \times 170 = 255 \mu\text{mho}/\text{cm}^2$ and that the membrane resistance should be $3900 \Omega \text{ cm}^2$. The result happens to agree closely with that of Fatt & Katz (1951) who obtained an average value of $4000 \Omega \text{ cm}^2$ in the sartorius muscle of *Rana temporaria*.

TABLE 8. Potassium conductances and permeabilities (from Table 7)

Row	Fibre reference	$[K]_o$ (m-mole/ kg H_2O)	$[K]_i'$	V (mV)	V'_K (mV)	$V - V'_K$ (mV)	Flow of K Inward = - Outward = + (pmole/ $\text{cm}^2 \text{ sec}$)	g_K ($\mu\text{mho}/\text{cm}^2$)	P_K (10^{-6} cm/sec)
1	j_1	95	228	- 31.7	- 22	- 9.7	- 333	3300	6.2
2	j_2	95	228	- 29.6	- 22	- 7.6	- 165	2100	3.9
3	j_3	95	228	- 27.0	- 22	- 5.0	- 97	1900	3.5
4	e	95	219	- 31.0	- 21	- 10.0	- 253	2400	4.7
5	c	10	104	- 66.8	- 59	- 7.8	- 37.2	460	4.9
6	d	10	113	- 69.0	- 61	- 8.0	- 50.6	610	6.4
7	a_1	10	113	- 71.5	- 61	- 10.5	- 32.6	300	3.2
8	l	10	127	- 72.3	- 64	- 8.3	- 46.6	540	5.5
9	h_1	2.5	100	- 70.4	- 92.9	+ 22.5	+ 14.3	60	1.3
10	h_2	2.5	84	- 62.3	- 88.4	+ 26.1	+ 9.15	34	0.7
11	a_2	2.5	113	- 62.4	- 96	+ 33.6	+ 11.7	34	0.6
12	m	2.5	104	- 67.6	- 94	+ 26.4	+ 5.8	21	0.4
13	r_1	2.5	125	- 77.0	- 98.5	+ 21.5	+ 18.5	80	1.7
14	r_2	2.5	125	- 110.0	- 98.5	- 11.5	- 22.2	190	5.5

For fibre diameters and values of $[Cl]_o$ see Table 7. V'_K is taken as the membrane potential when the fibre was in equilibrium with the solution. $[K]_i'$ was calculated from V_K by eqn. 2. The tonicity of the 95 mM-K solution was 1.75 times that of Ringer's fluid so that $[K]_i'$ is high. P_K was calculated by eqn. 20.

Potassium permeabilities and conductances

Since the flows of K and Cl through the membrane must be equal it is a simple matter to convert Table 7 (chloride movements) into a similar table (8) giving the effect of concentration and potential on potassium movements. The potassium conductance g_K is calculated by eqn. 6 and the potassium permeability P_K by

$$P_K = M_K \frac{RT}{VF} \frac{\exp(VF/RT) - 1}{[K]_i \exp(VF/RT) - [K]_o}, \quad (20)$$

when $M_K (= I_{K/F})$ is the net flow of potassium out of the muscle fibre. These results are given in Table 8; more complete but less direct information is given in Table 9, the method in this case being as follows. From the definitions of P_{Cl} and P_K given in eqns. 18 and 20 and the equality of M_K and $-M_{Cl}$ it follows that

$$V = \frac{RT}{F} \ln \frac{P_K [K]_o + P_{Cl} [Cl]_i}{P_K [K]_i + P_{Cl} [Cl]_o}. \quad (21)$$

This is the usual constant field equation (Goldman, 1943), but, in the present instance, the equation will be regarded as a necessary consequence of the definitions of P_K and P_{Cl} rather than as a deduction from a physical model. The relevance of the equation is that since P_{Cl} remains approximately constant it is possible to determine P_K from changes in membrane potential. The method was to assume that P_{Cl} had a constant value of 4×10^{-6} cm/sec, to calculate $[K]_i$ and $[Cl]_i$ from the potential when the fibre is at equilibrium in a given solution and finally to determine P_K from the new membrane potential when $[K]_o$ or $[Cl]_o$ were changed suddenly to a new value.

At low $[K]_o$ the argument is complicated by the fact that the membrane does not discriminate perfectly between K and Na. A simple but somewhat unrealistic approach which was used in Table 8 was to neglect $[Na]_o$ and to take a value of $[K]_i$ which agreed with the resting potential. An alternative was to allow for imperfect exclusion of Na by the equation

$$V = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}. \quad (22)$$

In applying the equation P_{Na}/P_K was taken as 0.01 (see p. 135) and $P_{Na}[Na]_i$ was neglected. This method was used in rows 15–25 of Table 9 but the results must be regarded as tentative, since changes in P_{Na} might introduce large errors. These reservations do not apply to the upper part of Table 9, which is considered fairly reliable.

For small displacements from equilibrium, for example in row 11, Table 9, the permeability ratio P_K/P_{Cl} was calculated from the estimates of transport numbers by the relation

$$\frac{P_K[K]_i}{P_{Cl}[Cl]_o} = \frac{g_K}{g_{Cl}} = \frac{T_K}{T_{Cl}}. \quad (23)$$

This relation follows from the definitions of conductance and permeability given in eqns. 5, 6, 18 and 20.

The conclusion from Table 9 is that the potassium permeability varies greatly with the force acting on the potassium ions. When $V - V_K$ is large and positive and potassium ions are moving outwards, P_K falls to a low value of about 0.05×10^{-6} cm/sec; when $V - V_K$ is negative and potassium ions are moving inwards P_K rises to about 8×10^{-6} cm/sec. The value under equilibrium conditions ($V = V_K$) is 1.2×10^{-6} cm/sec. The reduction of potassium permeability when the driving force acting on K^+ is outwardly directed agrees with Adrian's (1958) observations on the efflux of ^{42}K from muscles loaded with KCl and with Katz's (1949) experiments on electrical rectification in muscles immersed in isotonic solutions of K_2SO_4 .

For comparison with electrical data it is interesting to express the results as conductances rather than as permeabilities. With a fibre in 95 mM-K ($V_K = -22$ mV) the conductance for inward potassium current at $V = -32$

mV is $3000\mu\text{mho}/\text{cm}^2$ while the conductance for outward potassium current at $V = +70$ mV is only $30\mu\text{mho}/\text{cm}^2$; the conductance at equilibrium is roughly $1000\mu\text{mho}/\text{cm}^2$. With a fibre in 2.5 mM-K ($V_K = -101$ mV), the resting potassium conductance is about $80\mu\text{mho}/\text{cm}^2$ and the conductance calculated for outward current at $V = +70$ mV is perhaps $15\mu\text{mho}/\text{cm}^2$. With low $[K]_o$, the calculated variation of g_K is much less than that of P_K because the decrease of P_K is offset by the constant field type of rectification. We have no reliable evidence about the way in which P_K varies in the region of membrane potentials in which the fibre develops tension.

TABLE 9. Potassium permeabilities from measurements of membrane potential

I	II	III			IV	V	VI	VII	VIII	IX	X	XI
		Equilibrated					Test					
Row	Fibre reference	$[K]_o$ (mM)	$[Cl]_o$ (mM)	V_{eq} (mM)	$[K]_o$ (mM)	$[Cl]_o$ (mM)	V (mV)			$V - V_K$ (mV)	P_K/P_{Cl}	P_K (10^{-6} cm/sec)
$[K]_o = 95\text{ mM}$												
1	<i>o</i>	95	214	- 22	95	0	+ 85			+ 107	0.014	0.06
2	<i>o</i>	95	214	- 22	95	3.0	+ 71			+ 93	0.01	0.04
3	<i>g</i>	95	108	- 21.5	95	3.6	+ 43			+ 65	0.023	0.09
4	<i>g</i>	95	108	- 21.5	95	30	+ 2			+ 24	0.09	0.36
5	<i>g</i>	95	108	- 21.5	95	108	- 21.5			0	0.4	1.6
6	<i>o, p</i>	95	214	- 22	95	214	- 22			0	ca. 0.25	ca. 1.0
7	<i>g</i>	95	108	- 21.5	95	214	- 28			- 6.5	1.1	4.4
8	<i>e</i>	95	3.6	- 20.5	95	214	- 30.8			- 10.3	1.9	7.6
9	<i>j</i>	2.5	214	- 100.5	95	214	- 31.7			- 9.7	1.9	7.6
$[K]_o = 50\text{ mM}$												
10	<i>n</i>	50	30	- 32	50	6	- 19.9			+ 12.1	0.19	0.76
11	<i>n</i>	50	30	- 32	50	30	- 32			0	0.34	1.36
12	<i>n</i>	50	30	- 32	50	120	- 44.9			- 12.9	0.6	2.4
$[K]_o = 10\text{ mM}$												
13	<i>n</i>	50	30	- 32	(10)	(30)	(- 38)			+ 40	0.06	0.24
14	—	10	120	- 62	10	120	- 62			0	ca. 0.3	ca. 1.2
15	—	2.5	120	- 92.8	10	120	- 72.6			$V - V_{K+\alpha Na}$ - 7.5	1.3	5.2
$[K]_o = 2.5\text{ mM}$												
16	<i>f</i>	95	214	- 19	2.5	3.6	+ 74			+ 170	0.01	0.04
17	<i>j</i>	95	214	- 22	2.5	214	- 22			+ 76	< 0.02	< 0.08
18	<i>n</i>	50	30	- 32	2.5	30	- 32			+ 65	< 0.01	< 0.04
19	<i>m</i>	10	120	- 61.8	2.5	120	- 65			+ 25	0.2	0.8
20	—	2.5	120	- 94.5	2.5	30	- 72			+ 22.5	0.2	0.8
21	—	2.5	120	- 94.5	2.5	60	- 83			+ 11.5	0.2	0.8
22	—	2.5	120	- 94.5	2.5	120	- 94.5			0	0.5	2.0
23	<i>b</i>	0.5	120	- 113.2	2.5	120	- 102.2			- 8.4	0.7	2.8
24	<i>r</i>	2.5	30	- 98.5	2.5	120	- 110			- 11.5	0.7	2.8
25	<i>h</i>	2.5	28	- 90	2.5	120	- 101.9			- 9	1.2	4.8

Columns III and IV give the external concentrations and V the internal potential at equilibrium. VI and VII give the external concentrations and VIII the internal potential immediately after a sudden change in concentration. The definition and method of estimating P_K are given in the text. Eqn. 20 was used for rows 1-14 and eqn. 22 for 15-25. V_K was calculated by eqn. 2 and $V_{K+\alpha Na}$ by eqn. 4 using $\alpha = 0.01$. P_{Cl} is taken as 4×10^{-6} cm/sec. Rows 5, 6, 11, 14 and 22 are based on measurements of the slope of the $V-[Cl]_o$ or $V-[K]_o$ relations. Bracketed figures in row 13 were interpolated. Where no fibre reference is given, average data have been employed. In row 9, $[K]_i$ was taken from Table 8 as 228 mM. In rows 23-25, $[K]_i$ was calculated from the resting potential in Ringer's fluid.

DISCUSSION

The equivalent circuit in Fig. 13 provides a convenient way of describing the effects of K^+ and Cl^- on membrane potential. The batteries V_K and V_{Cl} represent the potassium and chloride concentration cells while R_K and R_{Cl} represent the resistance of the channels through which these ions pass. As the muscle comes into equilibrium with a new solution, KCl moves across the membrane until the e.m.f.'s of the two batteries are equal; electrically this corresponds to one battery being charged or discharged by the other. Under most conditions the change in e.m.f. is mainly in the chloride battery, which should therefore be regarded as having a much smaller 'capacity' than the potassium battery.

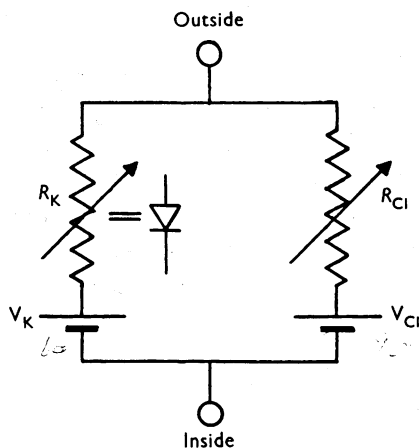


Fig. 13. Circuit diagram representing resting membrane of muscle. The rectifier element represents the variation of potassium permeability observed in the present experiments; the variation is in the same sense as that reported by Katz (1949). For a fibre equilibrated in Ringer's fluid $R_K \doteq 10,000 \Omega \text{ cm}^2$ and $R_{Cl} \doteq 5000 \Omega \text{ cm}^2$.

When the fibre is not in equilibrium, the membrane potential depends on the relative permeability of the two ions. Since the potassium channel acts like a rectifier which cannot pass much outward current, the membrane potential is determined by the battery with the smaller e.m.f. A more precise statement of the principle, using the internal potential, V , is that $V \doteq V_K$ if $V_K > V_{Cl}$ and $V \doteq V_{Cl}$ if $V_{Cl} > V_K$. In using the equivalent circuit one should remember that neither g_{Cl} nor g_K can be regarded as constant. In the case of the chloride channel, the variation of conductance with concentration and potential seems to be of the simple type expected from the constant field theory. This is not true for the potassium channel in which the conductance for inward currents may be 100 times that for outward currents, in spite of the fact that the internal concentration is greater than the external concentration. At present neither the physiological significance nor the physical nature of this rectification

is understood. The matter is particularly puzzling because the rectification is in the opposite direction to that required to explain the recovery of potential and the loss of potassium during the impulse. Presumably the behaviour of the membrane at short times is different from that in the steady state but on this point there is little experimental evidence.

In spite of uncertainties about the system controlling potassium permeability in muscle, the present experiments provide strong support for the conventional theory of the resting potential. Both K^+ and Cl^- have been shown to affect the membrane potential and the observed variations agree quantitatively with the idea that these two ions control the membrane potential. As is to be expected from the theory of Boyle & Conway (1941), changes in chloride concentrations at constant osmotic pressure and at constant $[K]_o$ produce only transient alterations of membrane potential, and the membrane potential at equilibrium depends mainly on the potassium concentration of the external medium. When $[K]_o$ is greater than 10 mM the potential is close to that of a potassium electrode; at lower concentrations it deviates in the manner expected from a slight permeability to sodium.

Since the average resting potential in Ringer's fluid, -94 mV, was less than the potassium equilibrium potential, -101 mV, one must suppose either that the fibres were slowly losing K and gaining Na or that a steady state is maintained by metabolic processes which absorb K and eject Na. The present experiments are probably consistent with either view, but, as there are other reasons for supposing that K uptake is not passive, it is simplest to assume that the passive leak of K is at least partly balanced by an active uptake driven by metabolism.

The approximate agreement between the resting membrane conductance calculated from our measurements and that found by Fatt & Katz (1951) has been mentioned on p. 152. A comparison can also be made with the experiments of Jenerick (1953) who measured the resistance across the membrane of fibres in the sartorius muscles of *Rana pipiens* which had been treated with solutions containing different concentrations of K and Cl. Making certain assumptions, Jenerick calculated the membrane resistance as $2500 \Omega \text{ cm}^2$ in Ringer's fluid (2.5 mM-K 116 mM-Cl), $290 \Omega \text{ cm}^2$ in 75 mM-K 189 mM-Cl and $170 \Omega \text{ cm}^2$ in 110 mM-K 224 mM-Cl. From his results one would predict a membrane resistance of about $200 \Omega \text{ cm}^2$ in 95 mM-K 214 mM-Cl. Taking our approximate values of $P_{Cl} = 4 \times 10^{-6}$ and $P_K = 1.6 \times 10^{-6}$ cm/sec (Table 9, row 5) and using eqn. 20, the membrane resistance of fibres equilibrated in 95 mM-K 214 mM-Cl is found to be $300 \Omega \text{ cm}^2$. Although agreement in order of magnitude is all that can be expected, it is perhaps worth pointing out that Jenerick's estimates in high K are likely to be too low since he assumed that the myoplasm resistance was not altered by raising the osmotic pressure and KCl content of the external solution. This does not apply to Jenerick's measure-

ments of the resting membrane resistance, but close agreement is not to be expected since the resting potential in 2.5 mM-K was 82 mV in his experiments and 94 mV in ours. In analysing his results, Jenerick assumed that the internal K and Cl concentrations did not vary with the osmotic pressure or KCl content of the external solution. It is therefore not surprising that his tentative conclusions about the relative magnitudes of P_K and P_{Cl} and about the way in which these permeabilities vary with membrane potential are very different from those in the present paper.

It is interesting to see whether some of the recent experiments on the effects of anions such as nitrate or iodide are consistent with the present results. Hutter & Padsha (1959) reported that NO_3^- or I^- increased the membrane resistance of the sartorius muscle about twofold. Using tracers, Abbott (quoted by Hill & Macpherson 1954) showed that I^- crossed the membrane less easily than Cl^- , and it is reasonable to infer that the same is true for NO_3^- . It has also been shown that the exit of Cl^- from the sartorius muscle is slowed by the presence of NO_3^- or I^- in the external medium (Harris, 1958 and R. H. Adrian, unpublished). All this suggests that NO_3^- or I^- cross the membrane less easily than Cl^- and that these anions retard the passage of Cl^- . Since K fluxes are not affected by NO_3^- or I^- (Edwards, Harris & Nishie, 1957), Hutter & Padsha concluded that the chloride conductance must normally be greater than the potassium conductance; this is clearly consistent with our conclusion that the chloride conductance accounts for $\frac{2}{3}$ of the total membrane conductance.

The interaction between NO_3^- and Cl^- explains an observation which otherwise seems inconsistent with the present results. Since NO_3^- crosses the membrane less easily than Cl^- one might expect that replacing Cl^- with NO_3^- would give a transient depolarization of the type observed with sulphate or with a Ringer's fluid in which some NaCl has been replaced by sucrose. In experiments with the sartorius muscle, Hutter & Padsha (1959) and R. H. Adrian (unpublished) found that the resting potential after equilibration was the same in NO_3^- as in Cl^- Ringer's fluid and they could detect no transient depolarization on first applying nitrate. Our experiments on single fibres showed either no change in resting potential or sometimes a hyperpolarization of a few millivolts when nitrate was applied. It therefore seems clear that although NO_3^- is less able to cross the membrane than Cl^- its action does not resemble that of SO_4^{2-} . An explanation can be given along lines similar to those followed by Hutter & Padsha (1959). In chloride media without NO_3^- we find $P_{Cl} = 4 \times 10^{-6}$ cm/sec. Hutter & Padsha's observations require a P_{NO_3} of, say, 1×10^{-6} cm/sec. If Cl and NO_3^- acted independently, one would expect that substituting NO_3^- would be equivalent to reducing $[Cl]_o$ to $\frac{1}{4}$ so there should be a depolarization of 20–30 mV. However, since NO_3^- reduces the flux of Cl without changing the resting potential, this ion evidently reduces P_{Cl} to some new value P'_{Cl} . If P'_{Cl} were 1×10^{-6} cm/sec, there would be no change in resting

potential on applying NO_3 and if P_{Cl} were less than 1×10^{-6} cm/sec there would be a transient hyperpolarization.

Since there is evidence of interaction in the passage of anions through the membrane it would not be surprising if the chloride fluxes calculated on the constant field theory differed from those observed experimentally. Taking the resting potential as -94 mV, P_{Cl} as 4.0×10^{-6} cm/sec and using the constant field theory, one obtains a chloride flux of about 40 pmole/cm² sec, as against the value of about 10 pmole/cm² sec calculated (Hodgkin, 1951) from the data of Levi & Ussing (1948). A more reliable comparison is for the case of a muscle equilibrated in Ringer's fluid plus 100 mM-KCl, where the value calculated with $P_{\text{Cl}} = 4 \times 10^{-6}$ cm/sec is 540 pmole/cm² sec and Adrian's (1958) results indicate a chloride flux of 200 pmole/cm² sec. The discrepancy is in the direction explained by movements of ions in single file along a chain of sites (Hodgkin & Keynes, 1955). A similar discrepancy is found with the potassium fluxes, the efflux in Ringer's fluid, calculated with $P_{\text{K}} = 2 \times 10^{-6}$ cm/sec, being 25 pmole/cm² sec as against 10 pmole/cm² sec found experimentally (Hodgkin & Horowicz, 1959). With a muscle equilibrated in Ringer's fluid plus 100 mM-KCl, the value calculated with $P_{\text{K}} = 1.2 \times 10^{-6}$ cm/sec is 120–240 pmole/cm² sec whereas Adrian's results give a potassium flux of about 100 pmole/cm² sec. In the case of chloride there may seem to be an inconsistency since net movements agree with the constant field theory whereas fluxes do not. However, one should remember, first, that agreement with the constant field theory was approximate and, secondly, that the single-file type of interaction may only become apparent in experiment with tracers. A long chain of sites, sparingly occupied by ions, ought to obey the constant field theory as far as net movements are concerned but would give anomalous fluxes if the ions could not pass one another.

The present experiments indicate that an appreciable quantity of chloride should enter a muscle fibre during an action potential. Taking the height of the action potential as 130 mV and its duration at half amplitude as 1.5 msec, taking the after-potential as 20 mV in amplitude and 30 msec in duration and using the constant field theory with a P_{Cl} of 4×10^{-6} cm/sec, one finds a chloride entry per impulse of 1.3 pmole/cm². This quantity is smaller than the apparent difference between the net entry of Na (about 15 pmole/cm²) and the net loss of K (about 10 pmole/cm²) associated with each impulse (Hodgkin & Horowicz, 1959).

One reservation which must be made about the present experiments is that nearly all the measurements were made on relaxed muscles and that we have not described the effects of solution changes which cause the muscle to contract. The primary reason for this omission is that it is much more difficult to obtain reproducible results on contracting muscles and that fibres were usually damaged irreversibly when they developed tension with the micro-electrode in

position. Certain aspects of the relation between membrane potential and tension will be described in a subsequent paper. For the time being all that need be said is that contractures induced by raising K do not have any obvious electrical sign and that the electrical effects of solutions which give tension are very much what one would expect from the results described here.

SUMMARY

1. The membrane potential of frog muscle fibres varied in the same manner as a potassium electrode when $[K]_o$ and $[Cl]_o$ were changed reciprocally at constant product, or if $[K]_o$ was altered in Cl-free solutions.

2. At constant $[K]_o$, variation of $[Cl]_o$ produced no permanent displacement of membrane potential, but there were large transient changes, lasting 10–60 min, in the direction expected for a chloride electrode.

3. At constant $[Cl]_o$, increasing $[K]_o$ from 2.5 to 10 mM produced a sudden depolarization and then a slow drift to an equilibrium value. When equilibrium had been established, reduction of $[K]_o$ from 10 to 2.5 mM produced only a small instantaneous repolarization and it took about an hour to establish the original resting potential.

4. The membrane potential of fibres which had been equilibrated with Ringer's fluid plus 93 mM-KCl was not sensitive to reduction of $[K]_o$ but varied in the same way as a chloride electrode when $[Cl]_o$ was reduced; the initial effect of removing all chloride was to make the inside of the fibre 70–80 mV positive to the outside.

5. Varying the internal chloride concentration, by altering the external osmotic pressure with sucrose, changed the membrane potential of a fibre loaded with Cl in the manner expected for a chloride electrode.

6. The results are explained quantitatively by assuming (i) a Donnan system of the Boyle-Conway type, (ii) K^+ and Cl^- are the ions which carry current through the resting membrane and (iii) the relative contribution of K^+ and Cl^- to the membrane potential depends on the direction in which the potassium ions are moving. The potassium permeability is fairly high (up to 8×10^{-6} cm/sec) for inward current but very low (down to 0.05×10^{-6} cm/sec) for outward current. The chloride permeability on the other hand remains at a constant value of about 4×10^{-6} cm/sec.

7. In a resting fibre equilibrated in Ringer's fluid the chloride conductance was about twice the potassium conductance; the absolute magnitude of the resting K and Cl conductances were about 100 and 200 μ mho/cm² respectively.

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